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Rotavirus G and P Types in Children With Acute Diarrhea in Blantyre, Malawi, From 1997 to 1998: Predominance of Novel P[6]G8 Strains

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One hundred rotavirus strains detected in children with acute diarrhea in Blantyre, Malawi, between July 1997 and January 1998 were characterized for G (VP7) and P (VP4) types by using multiplex, heminested, reverse transcription–polymerase chain reaction. A novel P[6]G8 rotavirus strain was identified in 42% of the specimens. The remaining strains comprised P[8]G3 (20%), P[6]G3 (10%), P[4]G8 (9%), P[6]G9 (3%), P[8]G4 (2%), P[6]G4 (2%), and P[4]G3 (1%). Rotavirus strains with mixed G or P types were identified in 2% of the specimens. Nine percent of the strains were nontypeable with the primers used. The P[6] genotype was identified in 57% of strains overall. This first description of serotype G8 rotavirus as a predominant strain has important implications for vaccine development in Africa. The finding of novel P/G combinations (P[6]G8 and P[4]G8) highlights the extraordinary diversity of rotaviruses in some countries. ***J. Med. Virol. 57:308–312, 1999.***

KEY WORDS: rotavirus; genotypes; Malawi

INTRODUCTION

Rapid progress in the development of rotavirus vaccines and their ability to prevent severe, life-threatening dehydrating diarrhea due to rotavirus, as demonstrated in recent field trials in both developed [Rennels et al., 1996; Joensuu et al., 1997] and developing [Pérez-Schael et al., 1997] countries, offers the prospect, through the routine vaccination of infants, of drastically reducing childhood morbidity and mortality caused by this virus. Two proteins, VP7 and VP4, together form the outer viral capsid and can segregate independently by the mechanism of reassortment. These proteins were used to establish a dual serotyping system whereby rotaviruses are classified according to VP7 (designated G since VP7 is a glycoprotein) and VP4 (designated P because VP4 is activated by protease cleavage) serotypes [Estes, 1996]. Since neutralizing antibodies to both VP7 and VP4 have been correlated with protective immunity, the epidemiology of these proteins has been extensively investigated.

The most common rotavirus G serotypes in fecal specimens from infants with diarrhea have been defined primarily by using enzyme-linked immunoassays with type-specific monoclonal antibodies, while genotyping methods, such as reverse transcription–polymerase chain reaction (RT-PCR) and hybridization with probes to genetically and antigenically distinct VP4 genes, have been used as surrogates for P serotyping because routine antigenic P typing methods have been more difficult to develop [Larralde and Flores, 1990; Coulson, 1993; Padilla-Noriega et al., 1993]. A revised classification scheme that accounts for both P serotypes (where available) and P genotypes has been adopted to facilitate description of the most common P types [Estes, 1996]. Studies from many countries worldwide utilizing these methods indicate that four rotavirus strains are of major epidemiologic significance, namely, types P[8]G1, P[8]G3, P[8]G4, and P[4]G2, with type P[8]G1 being by far the most prevalent [Gentsch et al., 1996]. A tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV) that incorporates the VP7 genes for serotypes G1, G2, G3, and G4 has been developed to provide protection against these important strains [Kapikian et al., 1996; Desselberger, 1998]. Recent studies, however, have suggested that rotaviruses of serotypes other than G1–G4 may predominate in some countries, including P[6]G9 strains in India [Ramachandran et al., 1996] and P[8]G5

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strains in Brazil [Gouvea et al., 1994a; Leite et al., 1996]. Non-G1–G4 strains may need to be incorporated into an effective rotavirus vaccine if surveillance indicates that the present vaccine is ineffective against them [Gentsch et al., 1996].

MATERIALS AND METHODS

The present study was based at the Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi. Malawi is situated in Central Africa and has a tropical climate, with a warm, wet season extending from November to April and a cooler dry season between May and October. Blantyre is the main commercial capital of Malawi and has a population of approximately 1 million people. The QECH is a government hospital that serves a predominantly urban population in Blantyre and surrounding areas. We examined rotaviruses collected between July 1997 and January 1998 from children under 5 years of age with acute, dehydrating diarrhea who either were given oral rehydration therapy (ORT) at the under-5 clinic at QECH (outpatients) or were admitted to the pediatric wards at QECH for ORT and/or intravenous fluid replacement (inpatients).

Rotavirus antigen was detected in feces by using an enzyme-linked immunosorbent assay (Rotaclone, Cambridge Biotech, Worcester, MA). Rotavirus double-stranded RNA was extracted with a glass powder method [Gentsch et al., 1992] and separated by polyacrylamide gel electrophoresis, followed by silver staining. Rotavirus G and P types were determined with a heminested, multiplex RT-PCR, using consensus and type-specific primers as described previously [Gouvea et al., 1990; Gentsch et al., 1992; Das et al., 1994].

Briefly, for G typing, consensus primers 9con1 and 9con2 were used in a 30-cycle RT-PCR after denaturation of the double-stranded RNA template; 2 μl of DNA product of this reaction was then used in a second amplification reaction (20 cycles) with 9con1 and type-specific primers 9T-1 (G1), 9T-2 (G2), 9T-3P (G3), 9T-4 (G4), and 9T-9B (G9). Since a large number of strains could not be typed by this method, full-length gene 9 PCR products were obtained from several nontypeable strains (which included strains with P[6] and P[4] genotypes) by using a degenerate mixture of primer pair beg9/end9 [Gouvea et al., 1990], made by mixing individual beg9 and end9 primers homologous to the VP7 proteins of G serotypes 1 to 4. Primers beg9-degenerate and end9-degenerate were then used separately to sequence the PCR products with the BIGDYE™ Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) after purification of the products by using the QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA). For each strain examined, comparison of partial gene 9 sequences (approximately 350 nucleotides for each primer) with sequence information available in the GenBank database demonstrated 98% to 99% identity at the nucleotide level to a serotype G8 Nigerian rotavirus strain, HMG89 [Adah et al., 1997a] (data not shown). On the basis of the published sequence of strain HMG89 and the sequence obtained here, an additional primer (MW-8, 5′-TCTTCA-AAAGTCGTAGTG-3′) was designed for use as a multiplex typing primer to allow PCR detection of type G8 strains (Fig. 1). The 9con1/9con2 product from nontypeable strains was used as the template, and the addition of primer MW-8 to the G typing primer cocktail generated PCR products of the expected size (661 bp) according to the nucleotide positions of 9con1 and MW-8. No cross-priming with MW-8 was seen for serotype G1, G2, G3, or G4 strains (Fig. 1). MW-8 was then used routinely as a multiplex primer to detect G8 strains in the fecal samples. For P typing, a similar strategy was employed by using consensus primers con3 and con2 in a first amplification RT-PCR (10 cycles), followed by a 30-cycle second amplification PCR using con3 and type-specific primers 1T-1 (P[8]), 2T-1 (P[4]), 3T-1 (P[6]), 4T-1 (P[9]), and 5T-1 (P[10]).

RESULTS AND DISCUSSION

A total of 100 isolates were examined (Table I). The distribution of strain types was similar in inpatients (n = 61) and outpatients (n = 39) (data not shown). Eight distinct strains were identified: strain P[6]G8 (42%), P[8]G3 (20%), P[6]G3 (10%), P[4]G8 (9%), P[6]G9 (3%), P[8]G4 (2%), P[6]G4 (2%), and P[4]G3 (1%). Strains with mixed G or P types were identified in 2% of specimens. A total of 9% strains could not be characterized for G type and/or P type. Serotypes G1 and G2 were not identified in any specimen.

This short study of rotavirus G and P types in Malawi has identified a remarkably diverse collection of strains, and several important observations require discussion.

First, the finding of a large number of serotype G8 rotaviruses was unexpected, since this serotype has not previously been considered to be of major epidemiologic importance in humans. The G8 strains reported here have a short electropherotype pattern. Rotaviruses possessing a “supershort electropherotype” were first reported in Indonesian children with acute diarrhea [Hasegawa et al., 1984], and one of these strains is the prototype human P4[10]G8 strain 69M [Matsuno et al., 1985]. G8 strains with long electropherotype patterns have since been recovered at low frequency from diarrheic infants in Finland and Italy [Gerna et al., 1990]. Serotype G8 has also been reported in studies from the United Kingdom [Beards and Graham, 1995] and Nigeria [Adah et al., 1997a]. A global distribution of G8 is likely, as seroepidemiologic surveys have demonstrated the presence of antibody to G8 in South American and Indian populations [Brussow and Sidoti, 1991; Kelkar et al., 1996]. As well as being isolated from children with diarrhea, G8 strains have also been detected in diarrheic cattle in Scotland [Snodgrass et al., 1990], Thailand [Taniguchi et al., 1991], Japan [Sato et al., 1997], and the United States [Gouvea et al., 1994a, 1994b]. Serotype G8 has also been detected in pigs [Gouvea et al., 1994], and a single equine serotype G8 rotavirus has been reported [Isa et al., 1996]. The origin of G8 strains in the human population is unknown,

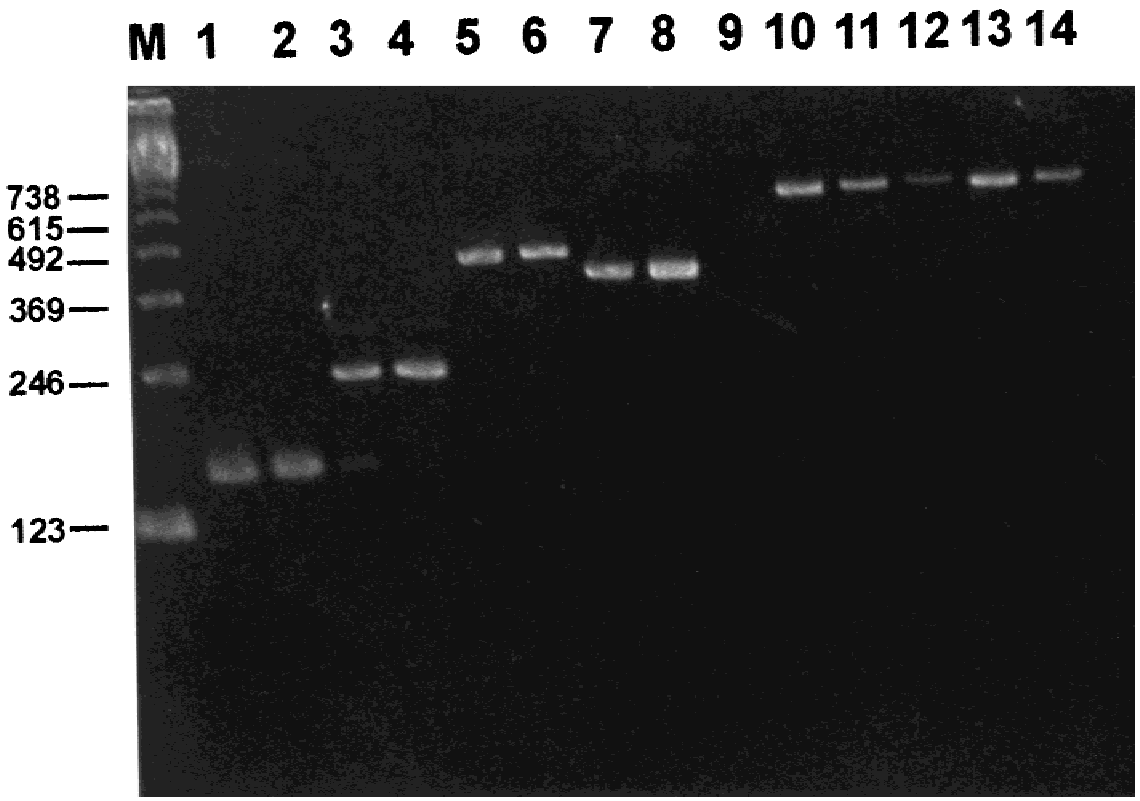

Fig. 1. Multiplex RT-PCR typing of rotavirus strains. Rotavirus double-stranded RNA was extracted from cell lysates or fecal specimens, 5 μl of the eluate was used for RT-PCR as described, and the products were analyzed on a 3% agarose gel [Gouvea et al., 1990]. Lane (M), 123-bp ladder (GIBCO-BRL, Long Island, NY), molecular weights are indicated on the left side. **Lanes 1–8**, second amplification products from fecal human rotavirus strains isolated in the United States and possessing serotypes G1 (lanes 1 and 2), G2 (lanes 3 and 4), G3 (lanes 5 and 6), and G4 (lanes 7 and 8); **lane 9**, second amplification product of negative control (water); **lanes 10–14**, second amplification products of five different Malawian G8 strains.

TABLE I. Rotavirus G and P Types in Children With Acute Diarrhea, Blantyre, Malawi, July 1997 to January 1998[a]

Genotype	G1	G2	G3	G4	G8	G9	G3 + G8	GNT[b]	Total (%)
P[4]	0	0	1	0	9	0	1	0	11
P[6]	0	0	10	2	42	3	0	0	57
P[8]	0	0	20	2	0	0	0	0	22
P[4 + 8]	0	0	1	0	0	0	0	0	1
P[NT][b]	0	0	6	1	0	0	0	2	9
Total (%)	0	0	38	5	51	3	1	2	100

[a]Rotavirus designations as recommended by the Rotavirus Nomenclature Working Group [Estes, 1996]. P[6]G8, P[4]G8 and P[6]G9 strains had short electropherotype patterns; the remaining strains had long electropherotypes.
[b]NT: nontypeable.

but interspecies transmission from a bovine source has been suggested [Browning et al., 1992; Gerna et al., 1994; Sato et al., 1997].

The finding of a high proportion of G8 strains in Blantyre may have important implications for rotavirus vaccine development. It is not known whether the current tetravalent vaccine, RRV-TV, which contains G serotypes 1 to 4, will provide cross-protection against G8 strains. If cross-protection is not provided, G8 would need to be included in an effective vaccine for areas where the prevalence of this serotype is high.

Second, rotavirus strains possessing the P[6]G8 genotype combination have not been previously recorded, and it is remarkable that this novel rotavirus strain was also the most commonly detected strain in Blantyre. This is also the first report of P[4]G8 strains; strains with P[4] genotype typically are type G2. Rotaviruses readily undergo reassortment in vivo and in vitro [Graham et al., 1987], and this is one mechanism whereby rotaviruses may evolve [Taniguchi and Urasawa, 1995]. The identification of rotaviruses with novel P/G combinations in Malawi emphasizes the ability of rotaviruses to undergo reassortment at high frequency [Unicomb et al., 1998] that may result in the formation of potentially important new strains.

Third, this study identified the highest proportion of

infants (57%) infected with rotaviruses possessing the P[6] genotype reported to date. Genotype P[6] strains have been well described in association with asymptomatic rotavirus infections of neonates [Gorziglia et al., 1988] but were considered uncommon pathogens in older infants with diarrhea [Steele et al., 1993]. However, 43% of children in a recent multicenter study of infants with diarrhea in India had P[6] infections [Ramachandran et al., 1996]. In addition, P[6] infections have now been reported at high frequency in infants with diarrhea in Nigeria [Adah et al., 1997b] and in the United States [Ramachandran et al., 1998]. Type P[6]G8 rotaviruses have also been detected in neonates without diarrhea in the pediatric high-care nursery at QECH in Blantyre (data not shown). The finding of this strain in asymptomatic neonates, and in a high proportion of diarrheic children from the same district, challenges the hypothesis that neonatal and community rotavirus strains are characteristically distinct [Tam et al., 1990].

Fourth, the finding of serotype G9 is of interest since the epidemiologic importance of this serotype has recently been demonstrated in India [Ramachandran et al., 1996], Bangladesh [Unicomb et al., 1998], and the United States [Ramachandran et al., 1998]. A recent report from Nigeria described dual infection with serotypes G1 and G9 in a single stool specimen [Adah et al., 1997c]. Further surveillance for G9 strains is warranted in Africa.

We were unable to type completely 9% of strains in this study, including six serotype G3 strains that could not be assigned a P genotype. Further investigation of these strains will be undertaken. Finally, the complete absence of serotypes G1 and G2 in Blantyre is in marked contrast to the global situation, where these strains are often the most common.

Limitations of this study are that strain characterization has thus far been restricted to only one city in Malawi (Blantyre) and surrounding areas, and only over a 6-month period of surveillance. It will be important to continue strain characterization in Blantyre and initiate studies in other regions of Malawi to determine whether this distribution of strains is widespread in the country and whether the strain pattern changes over time [Beards et al., 1989]. Similar studies in other African countries will provide crucial information for future vaccine programs in a continent where the need for an effective rotavirus vaccine is probably greatest [Cunliffe et al., 1998].

Finally, the G8 strains identified by RT-PCR and partial sequence analysis in this report have not yet been characterized by neutralization. Because of the potential importance and novel characteristics of these strains, a full antigenic and genetic characterization will be undertaken to better understand their relationship to other G8 strains and to common short electropherotype strains (e.g., subgroup I, P[4]G2, short electropherotype) strains.

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